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Identification of differentially expressed genes in the larval midgut of the silkworm (*Bombyx mori*) after bacterial infection

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Abstract: As the frontline encountering various microbes, intestine plays critical roles in insect immune responses. In order to investigate the immune responses in the silkworm midgut, we identified 18 differentially expressed genes after oral infection by *Pseudomonas aeruginosa* and *Staphylococcus aureus* using annealing control primer (ACP) based reverse transcriptional PCR technique. The expression profile of four genes during the first 24 hours post infection was monitored by quantitative real-time PCR. The results showed that the peptidoglycan recognition protein L1 (PGRP-L1) gene and a serine protease precursor gene were up-regulated by *P. aeruginosa* infection exclusively, while the 30kP protease A precursor gene was up-regulated significantly by both *P. aeruginosa* and *S. aureus* infection. Our study identified genes involved in recognition of invading bacteria and immune signaling pathways in the larval midgut of the silkworm after oral infection and may give some clues for further functional investigation of these genes.

Key words: *Bombyx mori*; midgut; bacterial infection; immune response; differentially expressed gene

1 INTRODUCTION

Insects have evolved elaborative and effective immune systems to deal with encountered microbes. By introducing bacteria into *Drosophila* body cavity with injection or pricking, it has been established that the production of antimicrobial peptides (AMPs) through Toll and IMD pathways functions as systemic immune reactions (Ferrandon *et al.*, 2007; Vallet-Gely *et al.*, 2008). Realizing ingestion as the main route for bacteria and other pathogens to enter into the hosts, researchers started to study the local immune defenses in the intestine after infection through the natural way, oral ingestion (Basset *et al.*, 2000; Schmidt *et al.*, 2008). The generation of reactive oxygen species (ROS) is an immediate defensive reaction to ingested pathogens. ROS in *Drosophila* reached the highest level at 3 h after natural infection with bacterium *Erwinia carotovora carotovora* 15 (*Ecc*15) (Ha *et al.*, 2005). ROS and nitric oxide are critical determinants of *Plasmodium* survival in *Anopheles gambiae* (Marois, 2011). In order to combat ROS-resistant pathogens, their hosts produce AMPs via IMD pathway in the gut as the second line of defense (Ryu *et al.*, 2006; Ryu *et al.*, 2010).

Little is known about gut immune reactions in insects other than the fruit fly and mosquito. Silkworm, *Bombyx mori*, has received much attention as a lepidopteran model insect in recent years. The transcript levels of key genes of the major immune pathways in silkworm were profiled systematically based on microarray after oral infection by pathogenic bacterium *Bacillus bombyseptieus* (Huang *et al.*, 2009). Toll-9 transcription was up-regulated in the silkworm gut after challenge with *Escherichia coli* or *Beauveria bassiana* in the diet (Wu *et al.*, 2010). Transcription of serine protease inhibitor (serpin) was also up-regulated in the midgut when the silkworm was infected by *B. mori* cypovirus (Wu *et al.*, 2011).

In this study, we used annealing control primer (ACP) PCR strategy to screen differentially expressed genes after the silkworm had been challenged with ingested bacteria and detected several inducible genes using quantitative real-time PCR. The efficiency of ACP technique and possible functions of these genes identified in this study were

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also discussed.

2 MATERIALS AND METHODS

2.1 Silkworm rearing and bacterial infection

B. mori (Nistari) larvae were reared on artificial diet (Fukui and Co., Ltd.) at 26℃ with 12 h light/12 h darkness photoperiod. When *B. mori* was at day 1 of the 5th instar, tetracycline (50 μg/larva) was added to the diet block (5 mm^3) to kill indigenous bacteria in the silkworm (Hamamoto *et al.*, 2004). *Pseudomonas aeruginosa* (PAO1) and *Staphylococcus aureus* were cultured in Luria-Bertani medium at 37℃ overnight. The cells were then collected by centrifugation at 5 000 g for 15 min, washed with PBS three times and resuspended with PBS to A_{600} = 20. Twenty four hours after the antibiotic treatment, 50 μL of the bacterial preparations or PBS (as the control) were added in the diet block or injected into the hemocoel to challenge the larvae. Each group includes 20 larvae. The animal survival was recorded in the following five days. For RNA extraction, 24 hours after the insects finished the diet blocks, 10 larvae from each group were dissected and the midgut tissues were collected and stored in Trizol reagent (Invitrogene) at −80℃.

2.2 Total RNA extraction and cDNA preparation

The midgut tissues were homogenized using a pellet pestle (Kontes) and total RNA was isolated using Trizol Reagent (Invitrogen) according to the standard protocol. The obtained RNA was purified using RNeasy MinElute Cleanup Kit (Qiagen) and the remaining genomic DNA contamination was removed by DNase treatment (Promega). Agarose gel (1.0%) was used to check the total RNA quality by loading 1 μL of total RNA samples. The concentration and A_{260}/A_{280} were determined by Biophotometer (Eppendorf). The purified RNA samples (3 μg) were used as the templates in the reverse transcription system of DEG GeneFishing Kit (Seegene) to synthesize the first-strand cDNA using dT-ACP1 primer (1.0 μmol/L). Reverse transcription was carried out for 1 h at 42℃ following the manufacturer's protocol. The first-strand cDNA was 5-fold diluted with water and stored at −20℃ for GeneFishing PCR.

2.3 ACP-based differential display RT-PCR

Differentially expressed genes were screened by ACP-based PCR using the GeneFishing DEG kits according to the manufacturer's protocol. Standard two-stage PCR was carried out in a final reaction volume of 20 μL. In the first stage, the second-strand cDNA was synthesized using the following thermal cycling parameters: one cycle at 94℃ for 5 min, 50℃ for 3 min and 72℃ for 1 min. The second-stage PCR was carried out to amplify cDNA using the following program: 40 cycles of 94℃ for 40 s, 65℃ for 40 s, and 72℃ for 40 s, followed by extension at 72℃ for 5 min. In our analysis, a total of 40 different ACP (annealing control primer) pairs were used. The amplified PCR products (5 μL) were separated on 2% agarose gels and stained with ethidium bromide. The remaining PCR products were stored at −20°C for TA cloning.

2.4 Cloning and sequencing of differentially expressed genes

Bands with significantly different intensity between control and infected groups were recorded and the rest of the PCR products were separated on 1.5% low-melting-point agarose gels (BioBasic Inc.). QIAquick Gel Extraction Kit (QIAGEN) was used to extract PCR products from the gels. The recovered PCR fragments were ligated with the TA cloning vector pMD-19 (TaKaRa) and transformed into *E. coli* JM109. The transformed bacteria were cultured for plasmid extraction using Plasmid Mini Kit (Omega) and sequencing (Shanghai Sunny Biotechnology Co.). All sequences were subjected to BLAST search for gene identification at Silkworm Genome Database (SilkDB: http://www.silkdb.org/silksoft/blast2-simple.html), GenBank (NCBI) and KAIKObase (http://sgp.dna.affrc.go.jp/KAIKObase).

2.5 Quantitative real-time PCR confirmation of the selected genes

To confirm the results of the ACP technology, quantitative real-time PCR was used to detect the amplification of four differentially expressed genes for A11, 30kP protease A precursor, PGRP-L1 and serine protease precursor. Total RNA was extracted from midguts of ten infected 5th instar larvae at 1, 2, 8, 16 and 24 h post infection (hpi) as well as from the uninfected larvae by Trizol reagent (Invitrogen). The RNA was treated with DNase I and purified using Direct-zol™ RNA Miniprep (Zymo Research). M-MLV Reverse Transcriptase (Promega) was used in the reverse transcription reaction following the manufacturer's instructions. Real-time qPCR was performed using the SYBR Premix Ex Taq™ Ⅱ Kit (TaKaRa) and Bio-Rad IQ5 Real-Time PCR system according to the manufacturer's protocol. Diluted first-strand cDNA (2 μL, 50 ng/μL) was used in each 25 μL reaction mixture and the final concentration of primers was 0.4 μmol/L. A reference gene *IF4A* was used to

normalize the samples (Wu *et al.*, 2010). Primers used in the real-time qPCR are displayed in Table 1. All the reactions were run in triplicate using the following thermal cycling parameters: denaturation at 95℃ for 30 s, followed by 40 cycles of 95℃ for 10 s and 53℃ for 30 s. Following amplification, melting curves were constructed to ensure the veracity of the experimental results (temperature range: 53 – 95℃). Standard curve of every gene was made to measure PCR efficiency, which was in the range from 0.9 to 1.1. R^2 values of the standard curves were greater than 0.997. Relative quantitative method (ΔΔCt) was used to evaluate the expression difference. In each time point, the expression level relative to the expression level of the control, which was arbitrarily set to one, was shown.

Table 1 Primers used in Quantitative real-time PCR for confirmation of differentially expressed genes

Protein	GenBank accession no.	Primer sequences (5′-3′)	Product size (bp)
A11	BGIBMGA004115	F: TTCCTTACCTGGAGAATG R: AACACAAATTGAAGTCACT	77
30 kP protease A precursor	BGIBMGA012788	F: CTCTTACCATAAGGATTAC R: CGATATTGTTGATTACGA	161
PGRP-L1	AK378516	F: GTGAAGTTATCTACGGAATGG R: ACAAATTGGGCTGACCTA	152
Serine protease precursor	BGIBMGA014427	F: GGATTCAGAATTAACACTAACAA R: GTCCGTGGATAATCATAGC	161
IF4A	BGIBMGA003186	F: AATCAACGTGAGCGTGAAGTG R: TCCCTTACGACCAAAACGTC	192

3 RESULTS

3.1 Mortality of silkworm to bacterial feeding and injection

First we compared the silkworm mortality to different routes of infection. Direct injection of the bacteria into the hemocoel caused the death of most silkworms in the test groups in two days, with PAO1 killing the worm faster than *S. aureus* (Fig. 1: A). Feeding infection showed slight pathogenic effect on the test silkworms (Fig. 1: B).

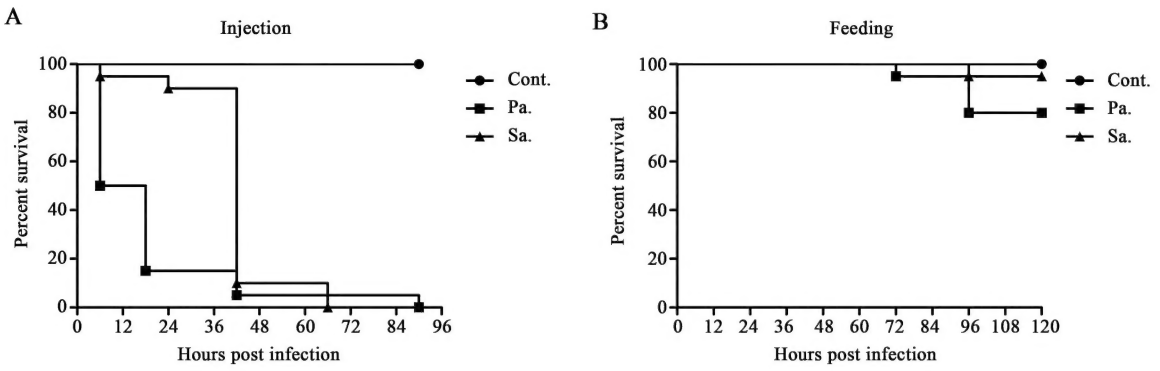

Fig. 1 Survival rate of silkworm infected by injection (A) and feeding (B) infection
Cont: Control (PBS); Pa: *Pseudomonas aeruginosa*; Sa: *Staphylococcus aureus*.

3.2 Identification of differentially expressed genes after bacterial infection

To identify genes with a predominant change of expression in the silkworm midgut after natural infection, we examined the gene expression using GeneFishing, a PCR-based differential display technique. RNA from midgut tissues infected with bacteria was individually subjected to ACP-based RT-PCR using a combination of two anchor Oligo (dT) primers (dT-ACP1 and dT-ACP2) and 40 arbitrary primers. PCR products were separated and visualized on 2% agarose gels. Several bands were defined as differentially expressed when there was a clear difference in intensity between infected and uninfected samples. A representative gel image is shown in Fig. 2. Totally, we identified 18 differentially expressed bands.

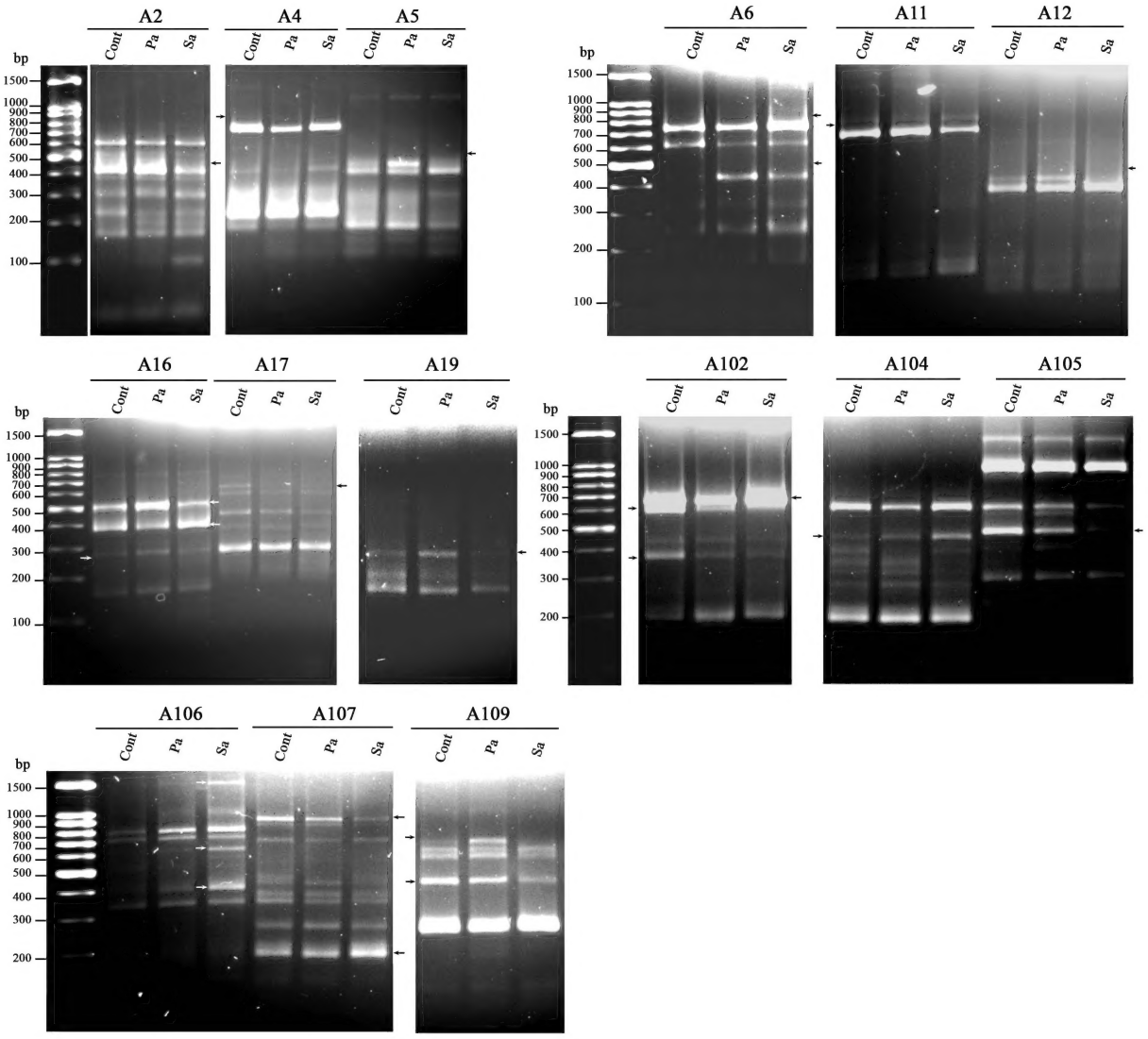

Fig. 2 Electrophoretogram of PCR products from the differential gene expression analysis

A2 – A109 are the primer pairs used in the GeneFishing PCR. Cont: Control (PBS); Pa: *Pseudomonas aeruginosa* infection; Sa: *Staphylococcus aureus* infection. The same for Fig. 3.

The differentially expressed bands were cut and subjected to sequencing. The DNA sequence of each differentially expressed gene was analyzed by BLAST search in the SilkDB, GenBank and KAIKObase. Cloning, sequencing, and BLAST search revealed 21 differentially genes (Table 2). Among them, A102c had no homolog in the databases; A11, A12 and A107b encoded proteins with unknown functions. Four genes encoding BGIBMGA004115, peptidoglycan recognition protein-L1 (PGRP-L1, AK378516), 30kP protease A precursor (BGIBMGA012788) and serine protease precursor (BGIBMGA014427), respectively, are of particular interest to us.

3.3 Confirmation of the ACP observations by real-time qPCR

Differential expression of the four selected genes was confirmed (Fig. 3). The expression of PGRP-L1 and serine protease genes was up-regulated exclusively by PAO1 infection. The expression of 30kP protease A precursor encoding gene significantly increased 2 – 16 h post PAO1 or *S. aureus* infection. BGIBMGA004115 responded to the infection quickly and dramatically. Its mRNA level increased about 5-fold at 1 h post infection, and then dropped about 1 000-fold during 2 – 8 hpi compared to the non-infected samples.

4 DISCUSSION

The intestinal barrier plays critical roles in host immune system. When the silkworms were infected by gut or hemocoel insults, we saw different survival results. Hemocoel infection caused the silkworm to die quickly. This implies that most bacteria acquired from diet have been killed in the gut before they pass the midgut barrier and enter the hemocoel. The other

Table 2　Annotation of the differentially expressed genes by the Blast search

Clone	SILK DB/GenBank/KAIKObase ID	E-value	Description of gene product	cDNA
A2	BGIBMGA004005	1.00E－108	Putative cuticle protein (Japanese oak silkworm)	AK378233
A4	BGIBMGA012788	0	30kP protease A (43k peptide) precursor	AK378510
A5a	BGIBMGA009130	1.00E－156	Ribosomal protein S20	AK386501
A5b	BGIBMGA004005	0	Putative cuticle protein (Japanese oak silkworm)	AK378233
A6a	BGIBMGA010979	0	Haemolymph juvenile hormone binding protein (JHBP)	AK388525
A6b	BGIBMGA012414	3.00E－61	Ribosomal protein L17	AK384926
A11	BGIBMGA004115	0	Unknown	AK388527
A12	BGIBMGA004115	1.00E－164	Unknown	AK388527
A16a	AB455925.1	0	Transposable element; non-LTR_TREST-W	AK381770
A16b	NM_001099806.1	1.00E－153	Small nuclear ribonucleoprotein polypeptide F	AK385097
A16c	AK378516	5.00E－95	Peptidoglycan recognition protein L1	AK378516
A17	AB473763.1	0	Transposon mariner-like element, non-LTR retrotransposon L1Bm	AK378418
A19	BGIBMGA009411	1.00E－29	Ribosomal protein S16	AK383780
A102a	BGIBMGA013756	0	Peritrophic membrane chitin binding protein	AK378243
A102b	BGIBMGA002635	0	Sugar transporter	AK383428
A102c	No hit	－	－	No hit
A104	BGIBMGA013413	1.00E－151	Tubulin alpha-1 chain	AK388384
A105	BGIBMGA010584	0	Chymotrypsinogen	AK378306
A106a	BGIBMGA010979	1.00E－142	Haemolymph juvenile hormone binding protein (JHBP)	AK388525
A106b	BGIBMGA009477	1.00E－174	Carboxypeptidase A	AK378467
A106c	BGIBMGA010979	0	Haemolymph juvenile hormone binding protein (JHBP)	AK388525
A107a	BGIBMGA009698	1.00E－125	Nascent polypeptide associated complex protein alpha subunit	AK383828
A107b	AK378032	2.00E－46	Unknown	AK378032
A109a	BGIBMGA014427	0	Serine protease precursor	AK378507
A109b	BGIBMGA000395	5.00E－77	Ribosomal protein L37	AK384780

possibility is that the bacteria were removed from the body through feces (Shao *et al.*, 2012).

To identify the candidate genes that might be involved in the silkworm midgut response to infection, we employed ACP-based PCR technique to identify differentially expressed genes in the larvae after bacterial infection. Through sequence analysis, we identified 18 genes responsive to oral infection. This technique uses high annealing temperature during amplification and, thereby, significantly diminishes artificial positives (Kim *et al.*, 2004). Additionally, detection of PCR products on standard ethidium bromide-stained agarose gels reduces the cost. These benefits make the method a popular one for identifying differentially expressed genes in various studies. These include plant defense-associated genes (Madala *et al.*, 2011), and genes associated with ovarian cancer (Kim *et al.*, 2010), metal metabolism (Han *et al.*, 2010), and host response to viral infection (Balasubramaniam *et al.*, 2011). This technique was also used in age estimation of forensically important blow fly (Boehme *et al.*, 2013) and insect immunity (Freitak *et al.*, 2009; Bang *et al.*, 2012). However, compared with microarray technique, this technique only makes a small number of differentially expressed genes visualized and recovered on agarose gels.

Insect gut immune system is composed of ROS generation and antimicrobial peptides production. In the current study, we identified some genes that are known to participate in insect immune responses. We confirmed that *B. mori* PGRP-L1 responded to gram-negative bacterium PAO1 infection. In *Drosophila*, PGRP-LC and PGRP-LE senses the DAP-type peptidoglycan containing bacteria and induces the IMD pathway (Charroux and Royet, 2012). *Manduca sexta* PGRP-1 recognizes certain bacterial strains and initiates prophenoloxidase activation pathway (Sumathipala and Jiang, 2010). When infected by means of injection, *B. mori* PGRP-L1 found to be induced by both gram-negative bacterium *E. coli* and

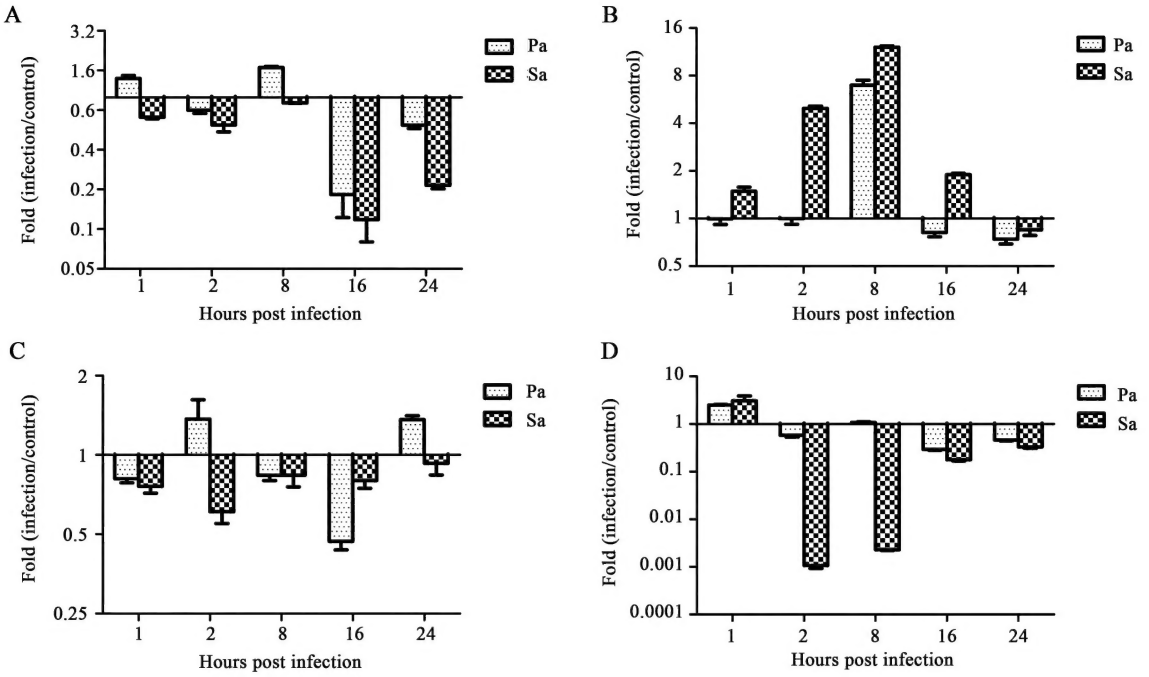

Fig. 3 Confirmation of four selected differentially expressed genes using quantitative real-time PCR

A: Serine protease precursor gene; B: 30kP protease A precursor gene; C: PGRP-L1 gene; D: BGIBMGA004115 gene. Data in the figure are mean ± *SE*.

gram-positive bacterium *S. aureus* (Tanaka *et al.*, 2008). Together with our finding, it appears that PGRP-L1 plays different roles in the midgut and the hemocoel.

We also identified two serine proteases in the silkworm midgut, which responded to the infection. One of them (BGIBMGA014427), a highly basic protease originally purified from the digestive juice, was thought to be involved in food digestion and cellular protein digestion during metamorphosis (Kotani *et al.*, 1999). Our results showed that its gene expression increases after the silkworm was challenged with PAO1. The other serine protease (30kP protease A) gene was up-regulated remarkably upon infection. Serine proteases are key components of the prophenoloxidase activation pathway and Toll pathway (Buchon *et al.*, 2009). Microarray and quantitative real-time PCR revealed that a group of serine protease genes were up-regulated by bacterial, fungal and viral infection (Zhao *et al.*, 2010). Functions of these serine proteases in the silkworm immune system need further investigation.

In summary, by using ACP-based differential expressed gene display technique, we identified 18 genes that might be involved in gut immune response in *B. mori*. Expression patterns of four selected genes were confirmed by quantitative real-time PCR. PGRP-L1 and two protease precursor encoding genes were up-regulated by oral infection of PAO1 and *S. aureus*.

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References

Balasubramaniam VRMT, Hassan SS, Omar AR, Mohamed M, Noor SM, Mohamed R, Othman I, 2011. Cellular transcripts regulated during infections with highly pathogenic H5N1 avian influenza virus in 3 host systems. *Virology Journal*, 8: 196 - 208.

Bang K, Park S, Yoo JY, Cho S, 2012. Characterization and expression of attacin, an antibacterial protein-encoding gene, from the beet armyworm, *Spodoptera exigua* (Hübner) (Insecta: Lepidoptera: Noctuidae). *Molecular Biology Reports*, 39: 5151 - 5159.

Basset A, Khush RS, Braun A, Gardan L, Boccard F, Hoffmann JA, Lemaitre B, 2000. The phytopathogenic bacteria *Erwinia carotovora* infects *Drosophila* and activates an immune response. *Proceedings of the National Academy of Sciences of the United States of America*, 97 (7): 3376 - 3381.

Boehme P, Spahn P, Amendt J, Zehner R, 2013. Differential gene expression during metamorphosis: a promising approach for age estimation of forensically important *Calliphora vicina* pupae (Diptera: Calliphoridae). *International Journal of Legal Medicine*, 127: 243 - 249.

Buchon N, Broderick NA, Poidevin M, Pradervand S, Lemaitre B, 2009. *Drosophila* intestinal response to bacterial infection: activation of host defense and stem cell proliferation. *Cell Host Microbe*, 5: 200 - 211.

Charroux B, Royet J, 2012. Gut-microbiota interactions in non-mammals: what can we learn from *Drosophila*? *Seminars in Immunology*, 24: 17 - 24.

Ferrandon D, Imler JL, Hetru C, Hoffmann JA, 2007. The *Drosophila* systemic immune response: sensing and signalling during bacterial and fungal infections. *Nature Reviews Immunology*, 7 (11): 862 - 874.

Freitak D, Heckel DG, Vogel H, 2009. Bacterial feeding induces changes in immune-related gene expression and has trans-generational impacts in the cabbage looper (*Trichoplusia ni*). *Frontiers in Zoology*, 6: 7 - 17.

Ha EM, Oh CT, Bae YS, Lee WJ, 2005. A direct role for dual oxidase in *Drosophila* gut immunity. *Science*, 310(5749): 847 - 850.

Hamamoto H, Kurokawa K, Kaito C, Kamura K, Razanajatovo IM,

Kusuhara H, Santa T, Sekimizu K, 2004. Quantitative evaluation of the therapeutic effects of antibiotics using silkworms infected with human pathogenic microorganisms. *Antimicrobial Agents and Chemotherapy*, 48(3): 774 – 779.

Han SH, Saminathan SO, Kim SJ, 2010. Insulin stimulates gene expression of ferritin light chain in osteoblast cells. *Journal of Cellular Biochemistry*, 111: 1493 – 1500.

Huang L, Cheng T, Xu P, Cheng D, Fang T, Xia Q, 2009. A genome-wide survey for host response of silkworm, *Bombyx mori* during pathogen *Bacillus bombyseptieus* infection. *PLoS ONE*, 4(12): e8098.

Kim YJ, Kwak CI, Gu YY, Hwang IT, Chun JY, 2004. Annealing control primer system for identification of differentially expressed genes on agarose gels. *Biotechniques*, 36(3): 424 – 434.

Kim YS, Do JH, Bae S, Bae DH, Ahn WS, 2010. Identification of differentially expressed genes using an annealing control primer system in stage III serous ovarian carcinoma. *BMC Cancer*, 10: 576 – 589.

Kotani E, Niwa T, Tokizane M, Suga K, Sugimura Y, Oda K, Mori H, Furusawa T, 1999. Cloning and sequence of a cDNA for a highly basic protease from the digestive juice of the silkworm, *Bombyx mori*. *Insect Molecular Biology*, 8(2): 299 – 304.

Madala NE, Molinaro A, Dubery IA, 2012. Distinct carbohydrate and lipid-based molecular patterns within lipopolysaccharides from *Burkholderia cepacia* contribute to defense-associated differential gene expression in *Arabidopsis thaliana*. *Innate Immunity*, 18(1): 140 – 154.

Marois E, 2011. The multifaceted mosquito anti-*Plasmodium* response. *Current Opinion in Microbiology*, 14: 429 – 435.

Ryu JH, Ha EM, Lee WJ, 2010. Innate immunity and gut-microbe mutualism in *Drosophila*. *Developmental and Comparative Immunology*, 34(4): 369 – 376.

Ryu JH, Ha EM, Oh CT, Seol JH, Brey PT, Jin I, Lee DG, Kim J, Lee D, Lee WJ, 2006. An essential complementary role of NF-kappaB pathway to microbicidal oxidants in *Drosophila* gut immunity. *EMBO Journal*, 25(15): 3693 – 3701.

Schmidt RL, Trejo TR, Plummer TB, Platt JL, Tang AH, 2008. Infection-induced proteolysis of PGRP-LC controls the IMD activation and melanization cascades in *Drosophila*. *FASEB Journal*, 22(3): 918 – 929.

Shao Q, Yang B, Xu Q, Li X, Lu Z, Wang C, Huang Y, Söderhäll K, Ling E, 2012. Hindgut innate immunity and regulation of fecal microbiota through melanization in insects. *Journal of Biological Chemistry*, 287(17): 14270 – 14279.

Sumathipala N, Jiang H, 2010. Involvement of *Manduca sexta* peptidoglycan recognition protein-1 in the recognition of bacteria and activation of prophenoloxidase system. *Insect Biochemistry and Molecular Biology*, 40: 487 – 495.

Tanaka H, Ishibashi J, Fujita K, Nakajima Y, Sagisaka A, Tomimoto K, Suzuki N, Yoshiyama M, Kaneko Y, Iwasaki T, Sunagawa T, Yamaji K, Asaoka A, Mita K, Yamakawa M, 2008. A genome-wide analysis of genes and gene families involved in innate immunity of *Bombyx mori*. *Insect Biochemistry and Molecular Biology*, 38: 1087 – 1110.

Vallet-Gely I, Lemaitre B, Boccard F, 2008. Bacterial strategies to overcome insect defences. *Nature Reviews Microbiology*, 6(4): 302 – 313.

Wu P, Wang X, Qin GX, Liu T, Jiang YF, Li MW, Guo XJ, 2011. Microarray analysis of the gene expression profile in the midgut of silkworm infected with cytoplasmic polyhedrosis virus. *Molecular Biology Reports*, 38: 333 – 341.

Wu S, Zhang X, Chen X, Cao P, Beerntsen BT, Ling E, 2010. BmToll9, an arthropod conservative Toll, is likely involved in the local gut immune response in the silkworm, *Bombyx mori*. *Developmental and Comparative Immunology*, 34: 93 – 96.

Zhao P, Wang GH, Dong ZM, Duan J, Xu PZ, Cheng TC, Xiang ZH, Xia QY, 2010. Genome-wide identification and expression analysis of serine proteases and homologs in the silkworm, *Bombyx mori*. *BMC Genomics*, 11: 405 – 505.

细菌感染后家蚕幼虫中肠差异表达基因的鉴定

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摘要：作为遭遇各种微生物的前线，昆虫的消化道在免疫反应中起到重要的作用。为了研究家蚕 *Bombyx mori* 肠道免疫反应，我们利用基于 ACP（annealing control primer）的反转录 PCR 技术，从中肠组织中鉴定得到 18 个在经口器感染绿脓杆菌 *Pseudomonas aeruginosa* 和金黄色葡萄球菌 *Staphylococcus aureus* 后的差异表达基因，并利用荧光定量 PCR 分析了其中 4 个基因在感染后 24 h 内的动态变化。结果表明：肽聚糖识别蛋白-L1（PGRP-L1）和一个丝氨酸蛋白酶前体基因特异性地受绿脓杆菌感染后上调；30kP 蛋白酶 A 基因受绿脓杆菌和金黄色葡萄球菌 2 种细菌感染后上调。我们的研究鉴定了经口器感染后家蚕中肠中参与入侵细菌识别和免疫信号途径的基因，可为对这些基因进一步的功能研究提供线索。

关键词：家蚕；中肠；细菌感染；免疫反应；差异表达基因

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